Chapter 13

PRODUCTION OF *ARTEMIA* IN CULTURE TANKS

Patrick Lavens and Patrick Sorgeloos

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I. INTRODUCTION

Compared to semi-intensive and intensive pond production techniques, super-intensive tank culture systems for *Artemia* offer two main advantages. First, there is no restriction with regard to production site or time; i.e., the culture procedure does not require highly saline waters or specific climatological conditions. Although *Artemia* in its natural environment only occurs at high salinities where no predators can survive (usually >100 g/l), they can thrive in natural sea waters, artificial sea waters, or saline well waters. Their physiological performance in terms of growth efficiency is even best in the salinity range 30—80 ppt.[1] Furthermore, optimal growth temperature can be maintained by the use of thermal effluents, or simply by heaters. The second advantage is that the controlled production can be performed with very high densities of brine shrimp, e.g., several thousand animals per liter vs. a maximum of a few hundred animals per liter in outdoor culture ponds. As a consequence very high production yields per volume of culture medium can be expected with tank rearing systems. However, it is obvious that in view of its operational sophistication and its energy and feed demand, this type of super-intensive *Artemia* culture yields a much more expensive *Artemia* product than with pond production. Nonetheless, they are a valuable alternative since more applications with *Artemia* culture in tanks occur in aquaculture as well as in the aquarium pet industry.[2,3]

Depending on the objectives and opportunities, different culture procedures for super-intensive *Artemia* production may be applied. In this regard we have divided this chapter into five main topics. The first part gives detailed information on abiotic and biotic culture conditions which may affect production yields. The second topic covers the design of upscaled culture systems and the general culture procedures. Disease control is explained in the third part, followed by harvesting techniques and production yields in the fourth topic. Last, we deal with the utilization of *Artemia* in aquaculture.

II. ABIOTIC AND BIOTIC CULTURE CONDITIONS

A. PHYSICO-CHEMICAL CULTURE CONDITIONS

1. Temperature and Salinity

Growth and survival of *Artemia* are greatly influenced by temperature and salinity.[4] Both parameters should be considered together since a reciprocal interaction has been detected for aquatic invertebrates in general[5] and is confirmed for *Artemia*.[6] Although it is generally accepted that brine shrimp are eurythermal as well as euryhaline, differences in survival occur among different strains. Nonetheless Vanhaecke et al.[7] state that for most strains (except e.g., in Chaplin Lake, Canada) a common range of preference of 19—25°C and 35—110 ppt can be detected in which mortality is less than 10%. Above 30°C temperatures soon become lethal for most strains. This is also the case for salinities under 20 g/l. The optimal range of temperature varies per strain if biomass production or food conversion efficiency is considered as the criterion: European bisexual strains and the population from San Francisco Bay, California (SFB) prefer the lower range (20—25°C) whereas parthenogenetic species and the Great Salt Lake, Utah (GSL) strain prefer the higher range (25—30°C).[8] See also Table 1.

2. Ionic Composition of the Culture Media

Besides natural sea water or brine, several artificial media with different ionic composition are frequently used in indoor installations for brine shrimp production. Examples of the composition of such media are given in Table 2. Sometimes *Artemia* grow better in these culture media than in natural sea water.[3,9] It is not even essential to use complex formulas; e.g., Dietrich and Kalle — media prepared with only six technical salts — proved

TABLE 1
The Effect of Temperature on Different Production Parameters for Various Geographical Strains of *Artemia*[a]

Geographical strain	Temperature (°C)					
	20	22.5	25	27.5	30	32.5
San Francisco Bay, CA						
Survival (%)	97	97	94	91	66	J
Biomass production (%)[b]	75	101	100	94	88	-
Specific growth rate[c]	0.431^{h}	0.464^{e}	0.463^{ef}	0.456^{f}	0.448^{g}	-
Food conversion[d]	3.89^{f}	3.35^{e}	3.64^{f}	3.87^{f}	4.15^{g}	-
Great Salt Lake, UT						
Survival (%)	77	85	89	89	87	88
Biomass production (%)	69	104	122	128	135	78
Specific growth rate	0.392^{i}	0.437^{g}	0.454^{f}	0.460^{ef}	0.465^{e}	0.406^{h}
Food conversion	3.79^{g}	2.90^{f}	2.65^{ef}	2.52^{e}	2.40^{e}	4.14^{h}
Chaplin Lake, Canada						
Survival (%)	72	75	77	65	50	-
Biomass production (%)	78	102	108	106	90	-
Specific growth rate	0.422^{g}	0.452^{ef}	0.459^{e}	0.456^{e}	0.437^{fg}	-
Food conversion	3.42^{e}	3.00^{e}	3.03^{e}	3.11^{e}	3.72^{e}	-
Manaure, Colombia						
Survival (%)	94	94	91	93	86	77
Biomass production (%)	52	102	112	110	104	105
Specific growth rate	0.379^{g}	0.451^{f}	0.462^{e}	0.460^{ef}	0.454^{ef}	0.455^{cf}
Food conversion	5.82^{f}	3.43^{e}	3.36^{e}	3.40^{e}	3.62^{g}	3.56^{e}
Buenos Aires, Argentina						
Survival (%)	96	90	94	91	80	-
Biomass production (%)	93	113	115	98	96	-
Specific growth rate	0.462^{f}	0.430^{e}	0.485^{e}	0.468^{f}	0.465^{f}	-
Food conversion	2.78^{e}	2.66^{e}	2.81^{e}	3.29^{f}	3.38^{f}	-
Larnaca, Cyprus						
Survival (%)	98	94	89	62	3	-
Biomass production (%)	61	94	95	83	0	-
Specific growth rate	0.384^{f}	0.430^{e}	0.432^{e}	0.417^{g}	0^{h}	-
Food conversion	4.29^{f}	3.23^{e}	3.43^{e}	3.93^{f}	-	-
Shark Bay, Australia						
Survival (%)	97	96	97	92	68	-
Biomass production (%)	48	67	84	75	66	-
Specific growth rate	0.332^{g}	0.369^{f}	0.393^{e}	0.381^{ef}	0.367^{f}	-
Food conversion	6.49^{g}	5.35^{ef}	4.57^{e}	5.08^{e}	5.81^{fg}	-
Tientsin, PR-China						
Survival (%)	95	94	91	93	84	54
Biomass production (%)	41	61	80	92	85	16
Specific growth rate	0.299^{g}	0.343^{f}	0.371^{e}	0.387^{e}	0.378^{e}	0.208^{h}
Food conversion	7.22^{g}	5.42^{f}	4.46^{ef}	3.84^{e}	4.22^{ef}	22.04^{h}

[a] data compiled from Vanhaecke *et al.*[8]
[b] expessed as % recorded for the *Artemia* reference strain (San Francisco Bay, batch 288-2596) at 25°C after 9 days culturing on a diet of *Dunaliella* cells.

[c] specific growth rate $k = \dfrac{\ln \frac{W_t}{W_o}}{T}$

with T = duration of experiment in days (=9)

[d] food conversion $= \dfrac{F}{W_t - W_o}$

F = µg dry weight *Dunaliella* offered as food
W_t = µg dry weight *Artemia* biomass after 9 days culturing
W_o = µg dry weight *Artemia* biomass at start of experiment

$^{e-i}$ means with the same superscript are not significantly different at the $P<0.05$ level.
J not analyzed.

TABLE 2
Artificial Sea Waters Used for Tank Production of *Artemia*[a]

Dietrich and Kalle[b]		Instant Ocean		ARC	
Solution A					
NaCl	23.9	Cl^-	18.4	NaCl	31.08
$MgCl_2.6H_2O$	10.83	Na^+	10.22	$MgCl_2$	6.09
$CaCl_2$ anhydric	1.15	SO^{2-}	2.516	$CaCl_2$	1.53
$SrCl_2.6H_2O$	0.004	Mg^{2+}	1.238	KCl	0.97
KCl	0.682	K^+	0.39	$MgSO_4$	7.74
KBr	0.099	Ca^{2+}	0.37	$NaHCO_3$	1.80
		HCO_3^-	0.142		
H_2O distilled	856	H_2O	1000	H_2O	1000
Solution B					
$NaSO_4.10H_2O$	9.06				
$NaHCO_3$	0.02				
NaF	0.0003				
H_3BO_3	0.0027				
H_2O distilled	1000				

[a] Data compiled from Lavens [3]; all data in g.

[b] Solutions A and B are prepared separately, then mixed and strongly aerated for 24 h.

to be as good as complete artificial seawater formulas. Moreover, culture tests with GSL *Artemia* in modified ARC (artificial sea waters) showed that KCl can be eliminated, and $MgCl_2$ and $MgSO_4$ reduced without affecting production characteristics. Ca^{++} concentrations >20 ppm are essential for chloride-habitat *Artemia* populations whereas carbonate-habitat strains prefer Ca^{++} concentrations <10 ppm in combination with low levels of Mg^{++} [10]

3. pH, Oxygen Concentration, and Illumination

In the literature, it is generally accepted that the pH tolerance for *Artemia* ranges from 6.5 to 8.[11,12]

With regard to oxygen, only concentrations <2 ppm can limit the production of biomass:[13] i.e., *Artemia* has the ability to adapt quickly to varying levels of O_2 by producing different hemoglobins with specific affinities for O_2.[13] For optimal production, however, O_2 concentrations >2 ppm are suggested.[14] High levels of O_2 (>5 ppm), on the other hand, will result in the production of pale animals, possibly with a lower individual dry weight, and which may therefore be less perceptible and attractive for the predators.

Little is known about the effect of light upon growth. Some studies postulate, without much proof, that *Artemia* is less active in darkness, thus consuming less energy for its metabolism and in this way allowing a faster growth.[15-17]

4. Water Quality

The quality of the culture medium can be affected by particles as well as soluble substances. Although no exact data on maximal concentrations are available, it is generally accepted that high levels of suspended solids will affect production characteristics, either by their interference at food uptake and propulsion by *Artemia*, or by bacterial growth and, consequently, oxygen demand. Furthermore, soluble waste products such as food proteins give rise to toxic nitrogen compounds (e.g., NH_3-N, NO_2^--N, and NO_3^--N) through oxidative deamination, mineralization, and nitrification processes. Ammonia is also excreted by the ammonotelic brine shrimp;[18] excretion rates, however, are a function of density, stage, and strain of *Artemia*, as well as temperature and food quality/quantity. The tolerance

levels in *Artemia* for NH_3-NH_4^+-N, respectively NO_2^--N, and NO_3^--N, seem to be much higher than in other water organisms: e.g., acute and chronical toxicity tests with GSL brine shrimp larvae showed no significant effect on survival (LC_{50}) nor growth for concentrations up to 1000 ppm NH_4^+, respectively 320 ppm NO_2^-.[3,19] It is therefore very unlikely that N-components will interfere directly with the *Artemia* cultures. Nevertheless, the presence of soluble substances should be restricted as much as possible because they are an ideal substrate for bacteria.

B. FEEDING CONDITIONS

Artemia is a continuous, nonselective, particle-filtering organism. The morphology and operation of the filter-feeding apparatus and alimentary canal of nauplii and (pre-)adults are described in detail in the chapter in this volume on morphology by Criel.

The coupling of propulsion, respiration, and filtration by the thoracopods results in a practically continuous filter feeding. Ingestion, however, can be interrupted at high particle concentrations: i.e., mandibles and maxillae stop their action, and the food bolus accumulated behind the labrum is rejected into the medium by the first pair of thoracopods.[20,21]

Furthermore, there is no selection in size or sorting of particles ingested by the brine shrimp.[1,22] Although maximal particle sizes that can be ingested by the naupliar and post-naupliar stages have not been exactly determined, diameters should not exceed 50 to 70 μm.

Artemia is an obligate particle feeder, at least with regard to fulfilling its protein and carbohydrate needs.[23] Micronutrients can be ingested under a soluble form only if concentrations in the culture medium are sufficiently high.

In spite of the above-mentioned characteristic of being a continuous filter feeder, various factors may influence the feeding behavior of *Artemia* by affecting the filtration rate, ingestion rate, and/or assimilation. As illustrated in Table 3, factors which may interfere are the quality and quantity of the food offered, the developmental stage of the larvae, and the culture conditions. More detailed information about these processes is given in Lavens[3] and in Coutteau and Sorgeloos.[30] In general we can make the following conclusions:

- A relatively wide range of particle densities is optimal for maximal ingestion; the minimal and maximal concentrations of this range, however, are very specific for each culture condition. Although the influence of these factors on the feeding activity of *Artemia* is only important when they measure extreme values, a combination of sub-optimal conditions that may occur in intensive culture systems might result in a limited rate of food uptake and consumption. This will eventually lead to low biomass production yields.
- The filter-feeding activity of brine shrimp is not very efficient in the early larval stages when only the second antennae contribute to filtration activity. Maximal filtration capacity is attained when all the thoracopods are functional.
- Artemia seems to be able to meet its protein requirements by regulating the food intake and assimilation as a function of the chemical composition of the diet offered.
- The food digestion process is dependent on the retention time in the gut which is determined by the ingestion rate, the enzyme activity, and the digestibility of the food.

C. SUITABLE DIETS

The nutritional requirements of brine shrimp can be summarized as follows: (1) the optimal protein: carbohydrate ratio is situated between 1:3 and 1:5;[31] (2) the essential amino acids are probably the same as for Crustacea in general; (3) exogenous nucleotides and sterols are essential;[32,33] (4) the essential vitamins are thiamine, nicotinamide, Ca-pantothenate, pyridoxine, riboflavin, folic acid, and putrescine;[23] (5) highly unsaturated fatty acids are not essential for growth but stimulate reproduction.[3,34]

TABLE 3
Filtration Rates and Ingestion Rates of *Artemia*[a]

Type of Food (diameter, dimensions or volume)	*Artemia* characteristics			Temp. (°C)	C_i	C_f	I_{max}	F_{max}	Ref.
	Strain	Stage	Size (mm)						
Yeast	—	Juvenile		20	1200		10.08		24
Yeast	—	Adult			1200		33.60		
Phaeodactylum (113 μm^3)	GSL		0.5	20	220	160			20
Phaeodactylum	GSL		1		180	90			
Phaeodactylum	GSL		2		210	100			
Phaeodactylum	GSL		5		120	55			
Phaeodactylum	GSL		7.5		145	70			
Phaeodactylum	GSL		10		160	15	9.6		
Dunaliella (345 μm^3)	GSL		10				3.6		
Chlorella (31 μm^3)	GSL		10						
Sand (3-40 μm^3)	GSL		9	20		1000	9.6		1
Phaeodactylum	GSL		9			50	13		
Chlamydomonas (10-15 $\mu m \times$ 6-9 μm)	SFB		5	28		120	7		26,27
Chaetoceros (46 μm^3)	—		11	20	400	400	14.4	408	21
Dunaliella (251 μm^3)	—		11		30	30	0.96	408	21
Chlamydomonas (1017 μm^3)	—		5	25	170		0.31		33
Chlorella (14 μm^3)	—		5		850		20.4		
Phaeodactylum	—	Nauplius		20			0.8		29
Phaeodactylum	—	Juvenile						17	
Phaeodactylum	—	Preadult						35-37	
Phaeodactylum	—	Adult						60-70	

Note: C_i, critical cell concentration for maximal ingestion (10^3 cells/ml); C_F, critical cell concentration for maximal filtration (10^3 cells/ml); I_{max}, maximal ingestion rate (10^6 cells/24 h/animal); F_{max}, maximal filtration rate (ml/24 h/animal); —, not specified.

[a] Data compiled from Lavens, P., Ph.D. thesis, 1989. With permission.

Subtle differences in nutritional requirements can be detected among the different geographical strains: e.g., the bisexual GSL *Artemia* grew optimal on the artificial test medium of Provasoli and Pintner[34] but the asexual population from Commacchio (Italy) was unable to perform well.

An enormous diversity of live and non-living diets is available as a potential food source for the successful culture of brine shrimp, e.g., live and dried algae, bacteria and yeasts (single cell proteins), and by-products from agricultural crops. Two specific characteristics of *Artemia* are correlated with this broad range in suitable diets: on the one hand the nonselective filtration behavior which facilitates the use of any type of food provided that the particle size is sufficiently small, and on the other hand the possibility to take up and digest exogenous microflora as part of the diet. Bacteria and protozoans which develop easily in the *Artemia* cultures are indeed able to biosynthesize essential nutrients as they use the supplied brine shrimp food as a substrate; in this way they compensate possible deficiencies in the diet composition. As early as 1956, Gibor[35] observed that *Artemia* grew better on live algae cultured under xenic as compared to axenic conditions. This effect is confirmed for diets based on dried algae or yeasts and fed to Anostracans or other invertebrates.[36-39] This phenomenon is even more critical with regard to the utilization of waste products from agricultural crops which on their own are probably nutritionally inadequate.

The interference by bacteria makes it difficult to identify nutritionally adequate diets as such, since growth tests are difficult to run under axenic conditions. As a consequence nutritional composition of the diet does not play the most critical role in the selection of foods suitable for high density culture of brine shrimp. More important criteria are

- Availability and cost
- Particle-size composition
- Digestibility
- Consistency in composition among different batches and storage capacity
- Solubility
- Food conversion efficiency (FCE)
- Buoyancy
- Possible interactions with the applied culture technology (e.g., clogging of the particles, fouling, etc.)

1. Live Micro-Algae

Many publications report on the culture success of *Artemia* fed with different algal species.[23] It is, however, useless to list all suitable algal species here as their nutritional composition (e.g., protein content) may fluctuate as a function of production conditions.[40,41] Moreover, in most cases the results obtained are from small culture trials and never from upscaled systems.

Some algal species are unsuitable as a diet for *Artemia* as a consequence of characteristics other than the nutritional composition: e.g., an indigestible, thick cell wall (*Chlorella*[42] or *Stichococcus*[35]), production of gelatinous substances which interfere with the ingestion mechanism (*Coccochloris*[44]), or secretion of toxic components (Dinoflagellates).[23]

Good examples of intensive *Artemia* productions using an algal diet are the St. Croix Artificial Upwelling Project at the U.S. Virgin Islands,[45] and a wastewater treatment system of the Dow Chemical Co. in Texas.[44] The main restriction, however, in using this food source is its limited availability. Mass culturing of suitable algae for *Artemia* is economically not realistic so that their use only can be considered in those places where the algal production is an additional feature of the main activity. In this regard, the effluents from intensive culture ponds of fish or shrimp, or from tertiary treatment plants may offer interesting possibilities for the super-intensive production of brine shrimp.[43]

2. Dried Algae

Various commercially available algal meals have been evaluated as a potential diet for *Artemia* culturing. In most cases they give satisfactory growth performance, especially when water quality conditions are kept optimal. To date, good culture success has been reported with dried *Scenedesmus*,[47] *Spirulina*,[48-50] *Gracilaria*,[51] and *Dunaliella salina*.[104] Drawbacks in the use of these feeds are their high cost (>$12/kg), as well as their high fraction of water soluble components which cannot be ingested by the brine shrimp but interferes with the water quality of the culture medium.

3. Bacteria and Yeasts

Single cell proteins (SCP) have several characteristics which make them an interesting alternative for micro-algae, not only for *Artemia* but also for other filter-feeding organisms, e.g., phyllopods, rotifers, bivalves, and penaeid shrimp:[30]

- The cell diameter is mostly smaller than 20 μm.
- The nutritional composition is fairly complete.[52]
- The rigid cell walls prevent the leakage of water soluble nutrients in the culture medium.
- Products are commercially available at acceptable cost (e.g., commonly used in cattle feeds[53,54]).

Nevertheless, these advantages do not guarantee culture success. Although good performances are reported for the small lab cultures of brine shrimp that are fed fresh or instant baker's yeast, or alcohol yeast, upscaling tests revealed highly variable production yields. When using different types of marine yeast, survival rates upon reaching the adult stage varied between 10 and 80%.[55,56] James et al. reported yields up to 7 kg of live weight *Artemia* when feeding marine *Candida* spp.[57] With *Rhodotorula*, *Saccharomyces* and *Kluyveromyces* species, low productions were obtained in raceway systems[58] or under flow-through conditions[3], due to low survival and growth performance after day ten. The problems that arise when feeding a yeast mono-diet are often assigned to nutritional deficiencies of the yeast diet[38,59,60] and therefore should be met by supplementation with other diets. Robin et al. detected that the addition of DL-methionine, choline, or a vitamin premix to methanol yeast resulted in a significant improvement of the *Artemia* growth.[61] Experiments in a flow-through installation where mono- and mixed diets containing *Kluyveromyces* yeast ("Protibel", France) were administered to high density cultures of GSL *Artemia* confirmed this hypothesis.[62] As can be seen from Figure 1 the high mortality that starts on day 10 could be overcome and better growth rates could eventually be obtained when 50% of the "Protibel" diet is supplemented with soybean pellets or corn (maize) bran. Biomass productions increased from 5 to >10 kg/m^3 and food conversion efficiencies remained very high as well, especially during the first 10 days (FCE = 0.6; in comparison FCE for micronized diets averages 1.0).[3] Analogous data were obtained by other researchers using mixed diets of yeast and waste products from agricultural crops.[61,63-65] These results indicate that SCP indeed could be interesting ingredients of diets used in the intensive production of *Artemia*.

In some trials SCP digestibility by the *Artemia* can be a problem as well: i.e. observations on feces of *Artemia* fed on baker's yeast revealed that they were packed with undigested cells. Complete removal of the complex and thick yeast cell wall by enzymatic treatment, or supplementation of the diet with live algae significantly improved assimilation rate and growth rate of the brine shrimp.[66] These experiments clearly demonstrated that the suitability of SCP (including micro-algae) is primarily related to the digestibility of the product. A digestibility treatment for fresh baker's yeast has recently been developed: the resulting diet can be successfully applied for the complete or partial substitution of microalgae in the diet of *Artemia* or mollusc larvae.[66-68]

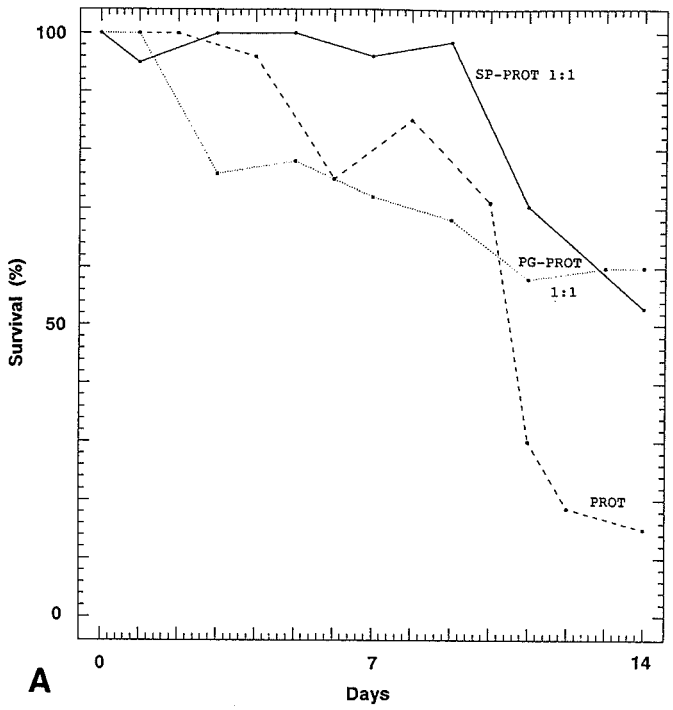

FIGURE 1. Survival (A) and growth (B) of Great Salt Lake *Artemia* cultured on Protibel-based diets. PROT, Protibel; SP, soybean pellets; PG, corn (maize) bran. (Modified from Lavens, P., Ph.D. thesis, 1989. With permission.)

According to D'Agostino[23] and Seki[69] live bacteria as a sole diet for *Artemia* seem to be unsuitable. Yasuda and Taga,[70] however, obtained good growth and survival when feeding them *Acinobacter* spp. Dried microbial detritus harvested from chitinovorous bacteria cultured on waste products, also supported good growth, but there was a low survival rate of the brine shrimp.[71]

4. Waste Products from the Food Industry

Nonsoluble waste products from agricultural crops or from the food-processing industry, e.g., rice bran, corn (maize) bran, soybean pellets, lactoserum, and sugarcane molasses appear to be a very suitable food source for high-density culturing of *Artemia*.[22] Their main advantages are the low cost and the worldwide availability. In most cases, however, these commercially available feeds do not meet the particle-size requirements for *Artemia* and an extra treatment is needed. When man power is cheap, a manual preparation which consists of blending the product in sea water followed by squeezing through a 70 μm filter bag can be used to obtain particle sizes less than 70 μm. Since this feed suspension cannot be stored, this manual method is restricted to daily preparation of the feed. This technique is successfully applied for brine shrimp culture in developing countries.[64,72] In order to reduce the manual labor in food preparations and to obtain a storable, dry product with the appropriate physical properties, mechanical techniques for dry grinding and processing need to be used. Of the different techniques examined to date, satisfactory results were only achieved with the relatively expensive micronization process: ± 80% (by weight) of the ground product has a particle size smaller than 70 μm.[3] Using micronized rice bran for *Artemia* culturing, food

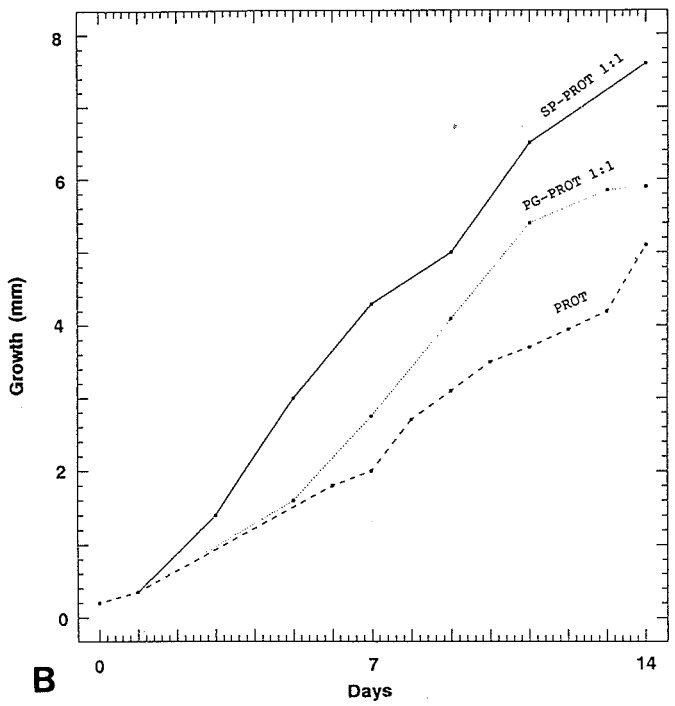

FIGURE 1B (continued)

conversion efficiencies of about 1 (ww *Artemia*/dw food) could be obtained with final productions up to 5 kg/m^3 or 25 kg/m^3 for batch[73] or flow-through conditions,[74] respectively.

Although many different waste products have been screened at the Artemia Reference Center (ARC) for application in intensive *Artemia* production, optimal results have only been obtained with diets consisting of a mixture of two ingredients: e.g., 1:1 mixtures of corn (maize) and rice bran or corn bran and soybean pellets (Figure 2). Comparison of the production data even show that there are no major differences in survival (± 60%) or body length (± 5.7 mm) among the feeding trials. This demonstrates that the biochemical composition of the diets is not of primary importance; e.g., the protein content is fluctuating between 18% and 40%. This is most probably due to the influence of microorganisms as previously explained. Comparison of the results of super-intensive production tests run at the ARC in the past decade and using waste products as diet, indicate that a high-fiber content in the food may be beneficial.

In conclusion, it may be stated that waste products from the food industry may be a good alternative to algae and SCP in the production of high densities of *Artemia*. Depending on the culture technique applied final yields can be in the order of 12 to 20 kg wet weight *Artemia*/m^3,[74,75] which is relatively close to the figures obtained with live algae.[45]

5. Microcapsules

Two publications mention the application of artificial diets under the form of microcapsules as a diet for *Artemia* culturing.[76,103] Such diets have the advantage that they should be more nutritionally complete and that no breakdown products pollute the culture medium. However, only low survival rates (20—35%) and moderate growth rates were attained after 18 d of culture.

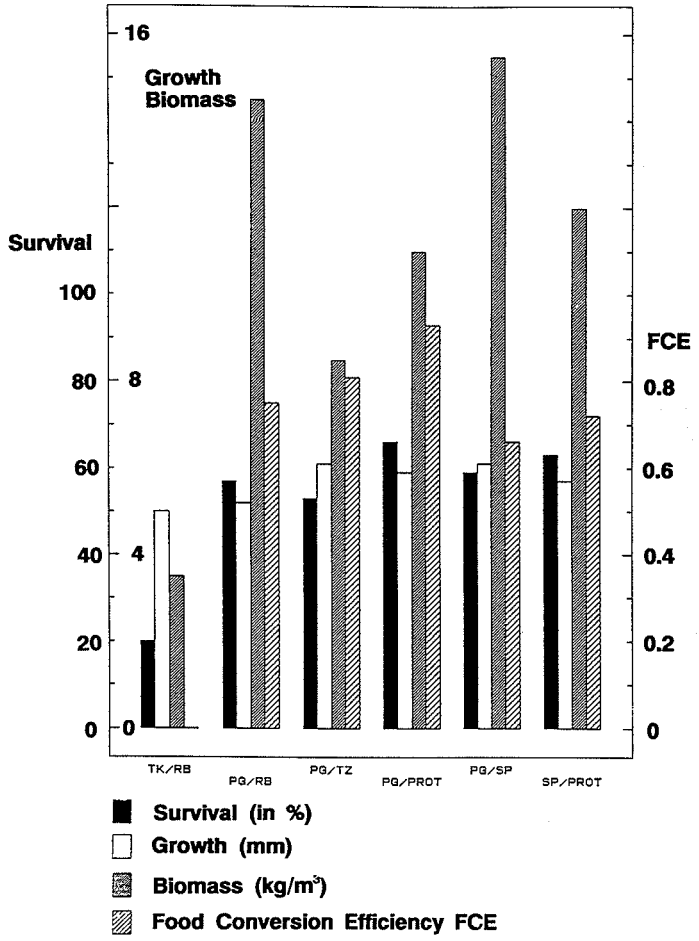

FIGURE 2. Production characteristics of super-intensive *Artemia* cultures fed different mixed diets. RB, rice bran; TK, wheat flour; PG, corn (maize) bran; PROT, Protibel yeast; SP, soybean pellets. (Modified from Lavens, P., Ph.D. thesis, 1989. With permission).

D. STRAIN SELECTION

Strain selection is not only important as a function of culture conditions (e.g., temperature; see Table 1), production yields can also vary from strain to strain. A comparative study with different *Artemia* strains fed two types of food (live *Dunaliella* algae and rice bran) demonstrated that differences in growth and survival may be important (see Tables 1 and 4). Under standard laboratory conditions GSL and Galerazamba, Colombia (GZ) brine shrimp produced about 50% more biomass than the reference strain from San Francisco Bay (SFB) origin. These results from small-scale experiments are confirmed in intensive production systems: highest biomass productions were always attained with GSL and GZ-populations cultured either in raceway systems[77] or in flow-through installations.[3,78]

E. DENSITY OF *ARTEMIA*

The growth rate of *Artemia* is not much affected by the density of animals in the culture

TABLE 4
Survival and Growth of Different *Artemia* Strains Cultured under Standard Laboratory Conditions with *Dunaliella* (DUN) or Rice Bran (RB) as Food for a Period of 7 Days[a]

Geographical strain	Survival at Day 7 (%)		Growth expressed as % recorded for reference strain San Francisco Bay (288-2596)	
	DUN diet	RB diet	DUN diet	RB diet
Larnaca Salt Lake (Cyprus)	70[c]		88[c]	
Santa Pola (Spain)	76[c]		88[c]	
Salin du Giraud (France)	66[c]		90[c]	
China (unknown locality)	84	83	91[c]	
Shark bay (Australia)	90(84)[b]	51[c]	95(96)	89[c]
San Francisco Bay (CA batch No 236-2016)	98		96	
Macau (Brazil, harvested in March 1978)	84(96)	90	96(98)	102
Barotac Nuevo (Philippines)	86	86	97	103
Tientsin (China)	96	86	98	
San Francisco Bay (CA batch No 933-235)	94		99	
Aigues Mortes (France)	90		99	
Izmir (Turkey)	96	88	101	108
Margherita di Savoia (Italy)	96	88	102	99
San Felix (Spain)	74[c]		102	
Macau (Brazil, harvested in May 1978)	94		103	
Bonaire (Netherlands Antilles)	66[c]	90	104	104
San Pablo Bay (CA batch No 1628)	90	92	105	106
Port Araya (Venezuela)	90	81	107	112
Eilat (Israel)	92[c]		107	
Lavalduc (France)	70[c]	93	109	96
Adelaide (Australia)	88(88)		113[c](113)	
Manaure (Colombia)	90	85	115[d]	129[d]
Bahia Salinas (Puerto Rico)	88	74[c]	122[d]	122[d]
Great Salt Lake (UT harvested in 1977)	94	86	119[d](125)	115[d]
Buenos Aires (Argentina)	72[c]	80	126[d]	124[d]
Galerazamba (Colombia)	98	87	126[d]	133[d]
Great Salt Lake (UT harvested in 1966)	66[c]		127[d]	
Chaplin Lake (Canada)	88	77	130[d]	130[d]

[a] Data compiled from Vanhaecke and Sorgeloos [9] and Vanhaecke *et al.*[8]
[b] In parenthesis result of replicate test.
[c] significantly different from the strain at the 0.01 level.
[d] significantly different from the strain at the 0.05 level.

medium. Depending on the culture technique applied, inoculation densities up to 5,000 larvae/l for batch culture,[73] 10,000 for closed flow-through culture,[75] and 18,000 for open flow-through culture[45] can be maintained without affecting survival and growth. These so-called maximum densities are in fact not critical as there is no real effect on behavior as is the case for most other anostracans, copepods, etc. However, each culture has its maximum carrying capacity: above these optimal densities, culture conditions become suboptimal (e.g., water quality deterioration and lower individual food availability). This results in reduced growth and increased mortalities during the second culture week (Table 5). Below the maximum carrying capacity there is no effect on biomass production but length and individual weight are significantly higher at lower densities.[3,73]

TABLE 5
Production Characteristics of Great Salt Lake *Artemia* Cultures as a Function of Different Animal Densities at Start of Culture[a]

Density (indiv. per ml)	Survival (%)			Growth (mm)			Biomass (in g wet weight/l)	Food Conversion Efficiency (ratio g dry food: g wet weight biomass)
	day 7	day 10	day 14	day 7	day 10	day 14	day 14	day 14
5	82	77	58	3.3	4.7	6.1	15.5	0.66
8	66	50	56	3.2	4.8	5.2	14.5	0.75
10	95	73	72	1.8	2.9	3.5	11.6	0.67
13	62	32	28	-	-	-	8.0	1.13
15	55	41	34	2.0	2.9	3.8	10.0	1.17
18	58	37	29	-	-	-	5.8	1.34
40	74	12	-	1.5	2.3	-	-	-

[a] Data compiled from Lavens.[3]

III. TECHNOLOGICAL ASPECTS OF MASS CULTURING

Although high-density culturing of brine shrimp is relatively simple compared to other aquatic organisms, several technological conditions are needed to ensure a functional culture system. First, an efficient agitation of the culture medium is essential for an even distribution of *Artemia* in the water column (and consequently a maximal utilization of the total culture volume), for a homogenous suspension of the food particles, and for a sufficient supply of oxygen in the medium. This agitation should not interfere with survival or growth. This is determined by the shape and surface/volume ratio of the tank. Different circulation systems have been described: paddle-wheels in shallow basins,[80] air-lines or aeration collars in high cylinders, rectangular tanks or cylindroconical recipients,[45,47,81] and air-water-lifts operating in a raceway concept.[77]

Optimal results have been obtained with rectangular tanks equipped with either an air-water-lift operating system (volumes up to 10 m^3) (Figure 3), or a central standpipe and aeration collar for tank sizes of maximum 1 m^3 (Figure 4).

The construction and operation of a raceway tank is simple. The most important parameter for the configuration of the tank is the height/width ratio, which should be close to 1/2 (Figure 5). If axial blowers are used for aeration, the water depth should not exceed 1.2 m to assure optimal water circulation. The corners of the tank may be curved to prevent dead zones where sedimentation can take place. A partition, to which air-water-lifts (AWLs) are fixed, is installed in the middle of the tank to assure a combined horizontal and vertical movement of the water which results in a screw-like flow pattern. AWLs are constructed with PVC pipes and elbows; the lower part of the tube which stands on the bottom of the raceway is cut at an angle of 45°, and the elbow's outflow is half-submerged (Figure 6).

An aeration line from a central air-distribution cylinder extends almost to the bottom of each AWL in order to assure optimal water lift effects. Although the agitation by this type of circulation systems is very intense, neither survival nor production of the brine shrimp are negatively affected.[14]

Second, the culture system should be designed in such a way that the most critical parameter, i.e., water quality, can be maintained as optimal as possible. This means that the concentration of particles and soluble metabolites should remain minimal so as to prevent toxicity problems, proliferation of micro-organisms, and interferences with the filter-feeding

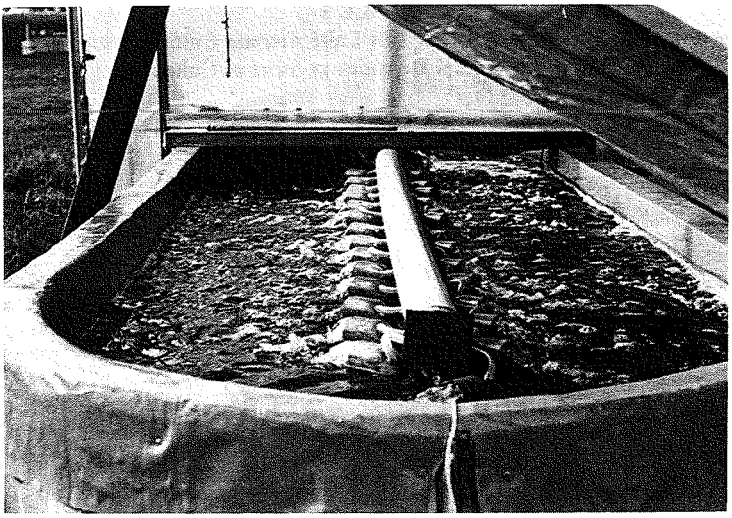

FIGURE 3. Air-water-lift operated raceway for super-intensive *Artemia* production following the stagnant culture principle (culture volume of 5 m^3).

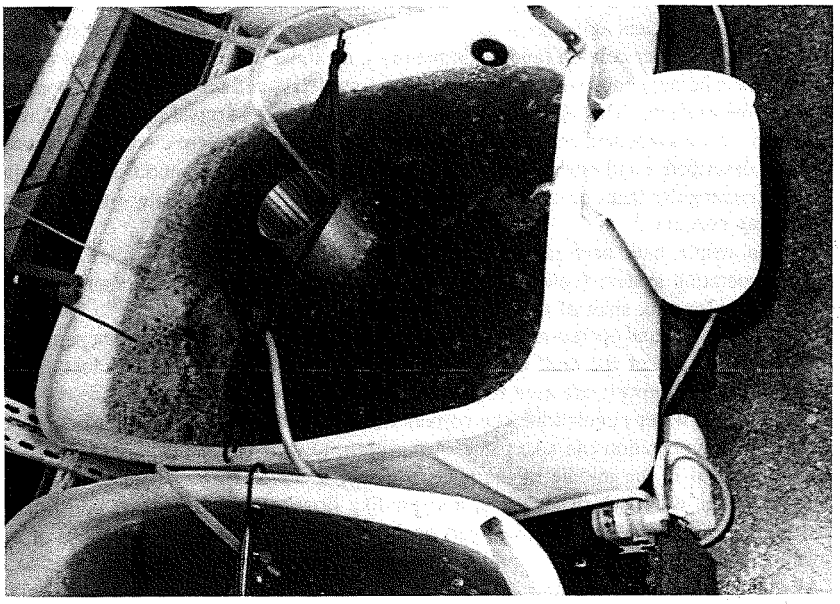

FIGURE 4. 300-l *Artemia* culture tank equipped with standpipe and aeration collar for super-intensive flow-through culturing.

system of the brine shrimp. Flow-through culture techniques are suitable for maintaining optimal water quality since water is (semi-)continuously renewed, whereas stagnant systems (batch) need a particle separation unit in order to reach sub-optimal levels of water quality.

Of the various techniques used for growing *Artemia* we will only describe those configurations that have already proven to be suitable for commercial exploitation or for inte-

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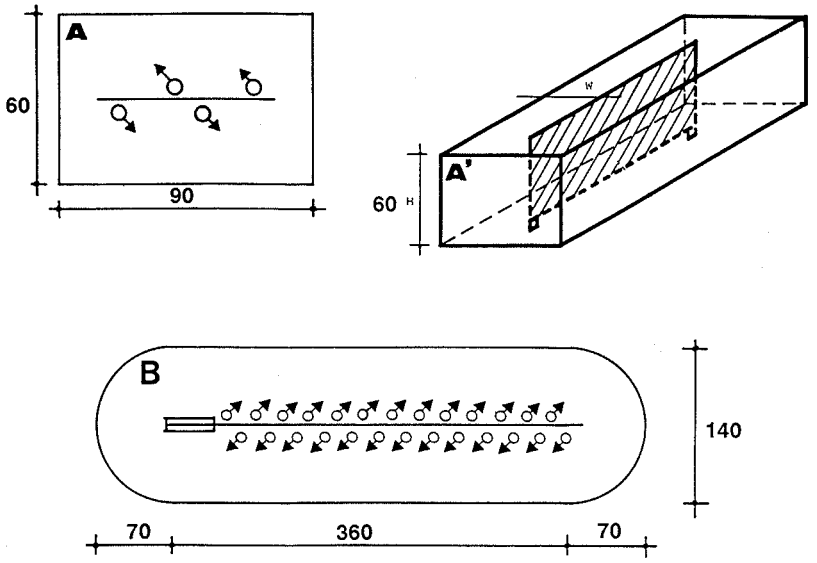

FIGURE 5. Schematic views and dimensions of raceway systems for *Artemia* culturing. (A) 300-1 tank: diameter AWL 4 cm, ± 7 l air/min/AWL;(B) 5 m^3 tank: 80 cm water depth, diameter AWL 5 cm, ± 16 l air/min/AWL. (Modified from Bossuyt, E. and Sorgeloos, P., *The Brine Shrimp Artemia*, Vol. 3., Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 133. With permission.)

gration into existing aquaculture activities. The type of installation will be subject to local conditions, production needs, and investment possibilities.

A. FLOW-THROUGH CULTURE TECHNIQUES

It is obvious that a discontinuous or continuous renewal of culture water, with consequent dilution of particulate and dissolved metabolites, will result in optimal culture conditions and thus high production capacities. Application of a flow-through culture technique, however, is limited to those situations where large volumes of sufficiently warm sea water (or brine) are available at relatively low cost — areas with (sub-)tropical climate or thermal effluents from power plants, desalination plants, and geothermal wells — and/or where large quantities of food are available, e.g., effluents from artificial upwelling projects, tertiary treatment systems, and intensive grow-out ponds of shrimp. These restrictions can also be overcome by the integration of a recirculation system which mechanically and biologically purifies the culture effluent so that finally it can be recycled to the *Artemia* tanks.

The most important and critical equipment in flow-through culturing is the special filter system used for efficient removal of excess culture water and metabolites without loosing the brine shrimp from the culture tank. Furthermore, it is essential that these filter-units operate without clogging for periods of more than 24 h.

Initially, standpipe filters were used which consist of a cylindrical PVC-frame around which an interchangeable nylon screen cylinder is fixed.[45,78] An aeration collar, positioned at the bottom of the filter, ensures a continuous friction of a curtain of air bubbles against the sides of the filter screen which results in an efficient reduction of filter-mesh clogging (Figure 7A). This filter has proven to be suitable for culture systems where live algae are fed to the *Artemia* population.[45]

However, when working with micronized waste products, we experienced much faster

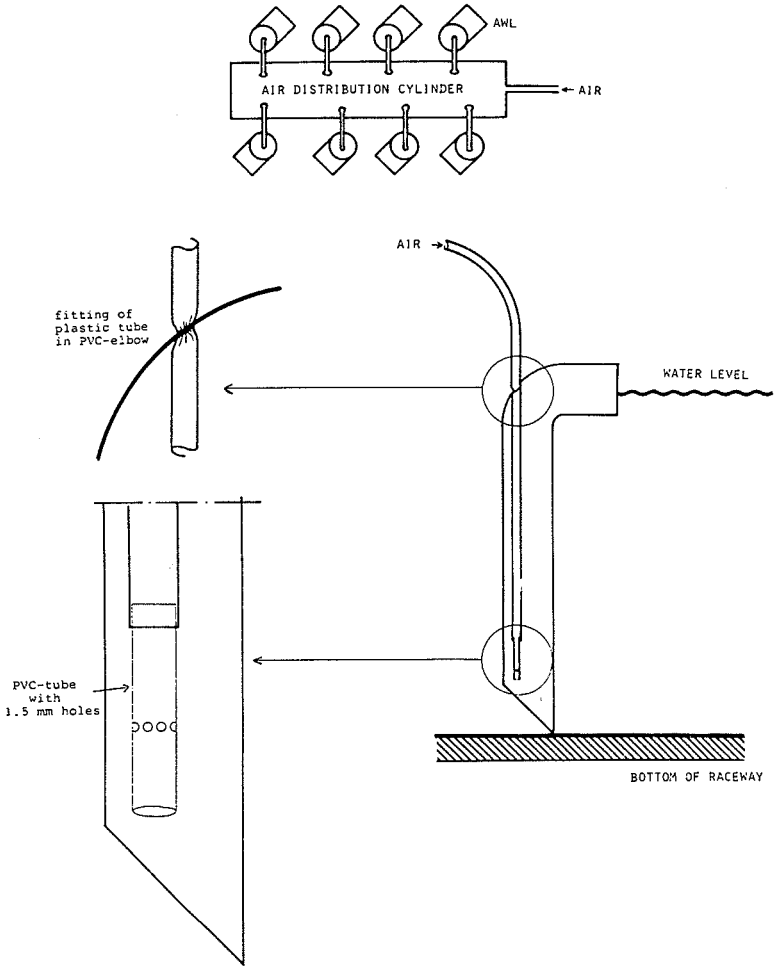

FIGURE 6. Details on the installation of AWL in raceways. (Modified from Bossuyt, E. and Sorgeloos, P., *The Brine Shrimp Artemia*, Vol. 3., Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 133. With permission.)

clogging of the filter screen, especially for the smaller mesh sizes, and the design of the standpipe had to be adapted.[74] A PVC-frame was constructed with all stand-up sides inclined under an angle of 10° and over which the filter-screen bag of a specific mesh size (depending on the size of the *Artemia*) can be fitted (Figure 7B). In this arrangement, the rising air bubbles have a very intensive contact with the screen, ensuring a more efficient cleaning.[74] The upper part of the filter bag which is positioned just above and underneath the water level is made of smooth nylon cloth to prevent any trapping of the cultured larvae that are foamed-off by the effect of the strong aeration.

A new type of cylindrical filter system (Figure 8) has recently been introduced for still more efficient removal of the effluents from brine shrimp culture tanks.[75] It consists of a welded-wedge screen cylinder, made of stainless steel and vertically placed in the center of the culture tank. Its bottom and top part extend into PVC-rings, and an aeration collar is

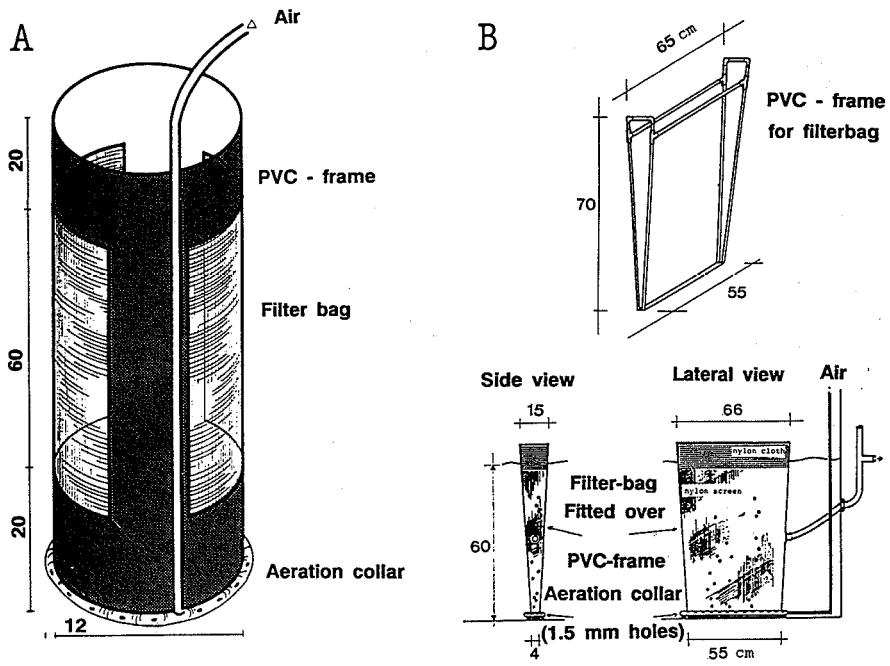

FIGURE 7. Schematic views and dimensions of filter systems used in flow-through culturing of *Artemia*. (Modified from Brisset, P., Versichele, D., Bossuyt, E., De Ruyck, L., and Sorgeloos, P., *Aquacult. Eng.*, 1, 115, 1982; Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, Artemia Reference Center, State University of Ghent, Belgium, 1986. With permission.)

fixed to the lower end of the filter. This welded-wedge system has several advantages in comparison to the filter-screen types:

- Oversized particles with an elongated shape can still be evacuated through the slit openings.
- The specific V-shape of the slit-openings creates specific hydrodynamic effects as a result of which filter particles that are only slightly smaller than the slit-opening itself are sucked through.
- Possible contact points between particles and filter are reduced to two instead of four, which consequently reduces the clogging probability.

Comparative tests in the ARC flow-through recirculation system[3,75] revealed that this new filter can be operated autonomously for much longer periods than with traditional mesh filters. Proportionally smaller welded-wedge filters can be used, leaving more volume for the animals in the culture tank. Furthermore, they are very cost effective since they do not wear out.

As brine shrimp grow, the filters are progressively switched for one with a larger mesh—or slit-opening—in order to achieve a maximal evacuation of molts, feces, and other waste particles from the culture tanks. A set of filters covering a 14-d culture period should consist of approximately six different slit/mesh-openings starting from 120 μm to 150 μm up to 350 μm and 550 μm, respectively (Table 6).

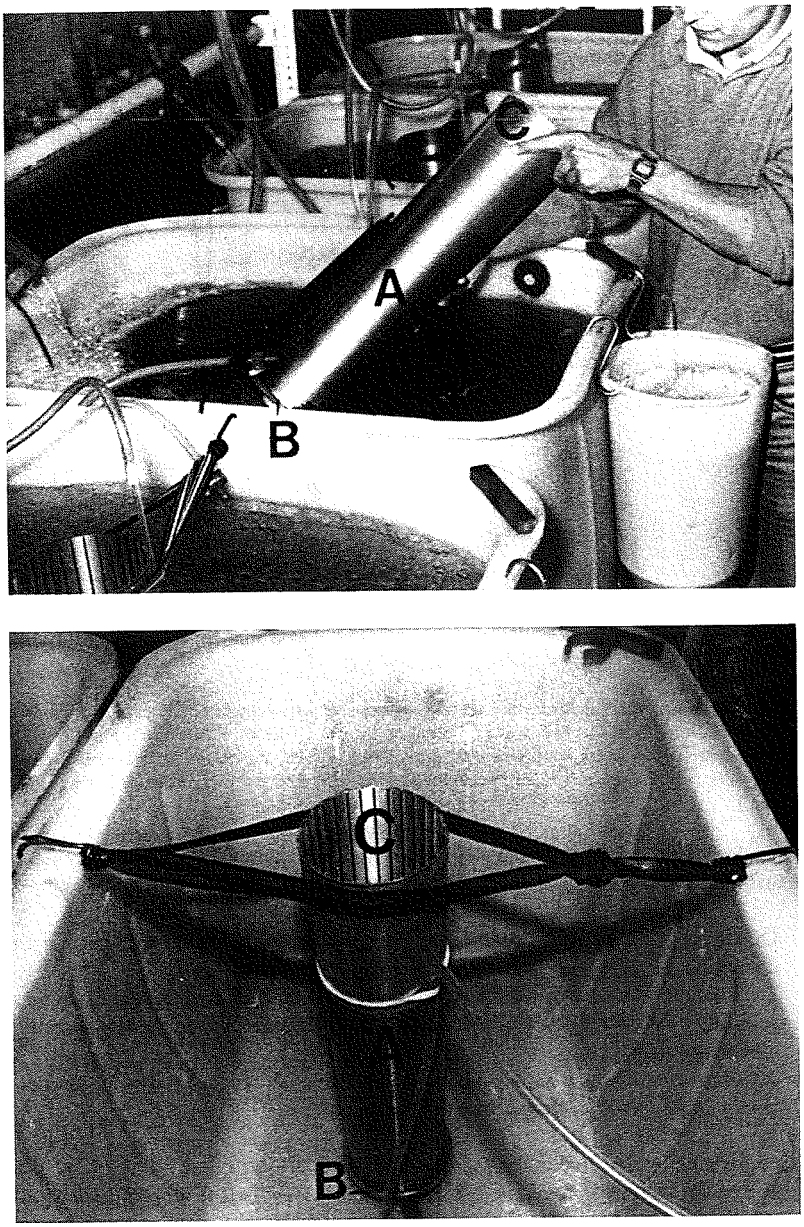

FIGURE 8. Overview of new filter system for use in flow-through *Artemia* culturing; (A) stainless-steel welded-wedge screen cylinder, (B) aeration collar, (C) upper PVC-ring.

The water-flow intake, and thus the hydraulic retention time in the culture tank, need to be adjusted in relation to the filter size used and the overall culture conditions in order to reach an optimal compromise between efficient evacuation of wastes and minimal food losses. An optimal regime applied for a 300-l rectangular tank (see Figure 4) equipped with welded-wedge filters of 65 cm high and 14 cm in diameter is outlined in Table 6. The

TABLE 6
Typical Example of Rate of Exchange of Filters, Culture Water Renewal and Feeding Regime Applied in a 300 L Super-Intensive *Artemia* Culture[a]

Culture day	Slit opening of filter (μm)	Flow rate (l/hr)	Retention time in culture tank (hr)	Feeding regime (period between distributions of 2.5 g each) (min)	Food added per day (g)
1	120	80	3.75	36	100
2	150	100	3	30	120
3 - 4	200	100	3	24	150
5 - 7	250	150	2	20	180
8 - 9	300	150	2	20	180
10 - 12	350	200	1.5	15	240
13 - 14	350	300	1	12	300

[a] Data compiled from Lavens.[3]

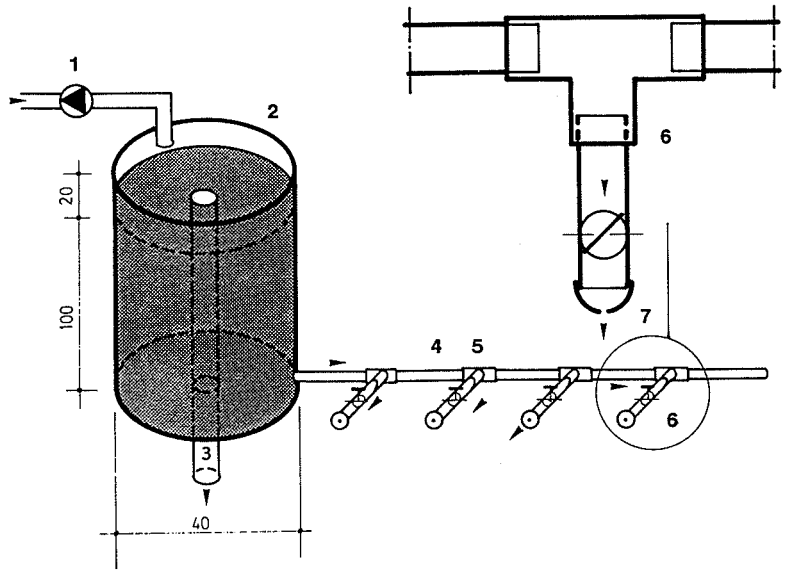

FIGURE 9. Schematic view of a water-inlet system assuring precise control of the flow rates to the culture tanks. (1) water pump; (2) constant head tank with (3) overflow; (4) supply pipe with taps (5); (6) PVC-caps with calibrated outlet opening (7). (Modified from Sorgeloos, P., Lavens, P., Léger, P. Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, Artemia Reference Center, State University of Ghent, Belgium, 1986. With permission.)

installment of the flow rate can easily be done by using interchangeable PVC-caps which fit onto the PVC-water-supply pipe connected to a constant head tower, and which have a calibrated outlet (Figure 9). Depending on the size of the installed outlets a preset flow rate can be administered.

Since the continuous water addition will dilute the amount of food in the culture tank (provided no food is taken in with the influent water), fresh food needs to be added frequently in order to maintain optimal food particle concentrations for growth. This can be achieved with water pumps connected to the feed tank and activated by a preset electronic timer. A properly functioning system for food distribution is outlined in Figure 10; its main component

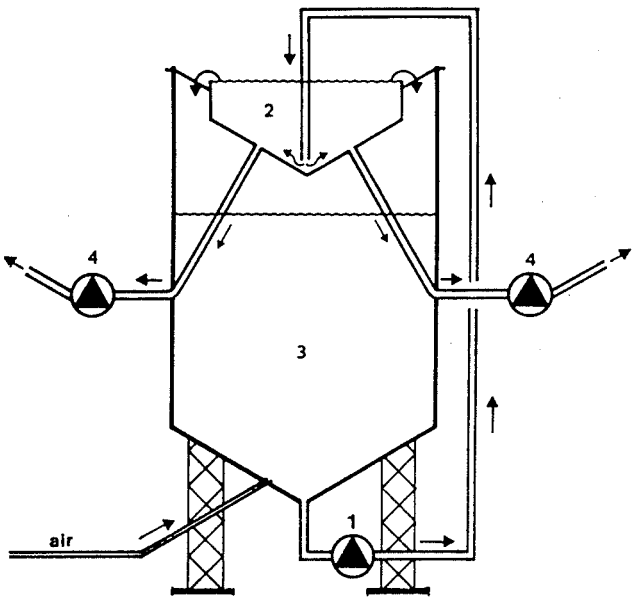

FIGURE 10. Schematic view of food distribution system for use in super-intensive *Artemia* culturing. Food is continuously pumped (1) into the constant head tank (2), the overflow of which drains into the main reservoir (3); individually activated by electronic timers, pumps (4) distribute feed suspension to the respective culture tanks. (From Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E., and Sorgeloos, P., *J. World Aquacult. Soc.*, 16, 498, 1985. With permission.)

is a small constant head which assures equal food additions at any moment of the day. Pumping rates are adjusted daily to maintain constant levels of food in the culture tank (Table 6).

Using agricultural by-products as the diet source, the transparency of the culture medium can be used as a very useful parameter for the determination of the optimal food dose.[22,77] For the corn (maize) bran/soybean pellet (4:1) diet, transparency levels of 20 to 25 cm appear to be optimal for juvenile *Artemia*.[75] The transparency measurements can easily be performed with a modified Secchi-disk.[77] A simple electronic transparency meter (Figure 11) can be connected to the effluent tube of the culture tank to monitor possible overfeeding conditions. When the food level drops below a preset critical value, the apparatus will shut off the food distribution pump. Accurate functioning of the transparency meter is ensured by the continuous water flow through the apparatus, together with a regular air injection close to the light emitter, which limits sedimentation and clogging.

However, for monitoring adult cultures of brine shrimp a different feeding strategy should be applied. Molting rates in older *Artemia* gradually decrease as a result of which the setae of the thoracopods become easily clogged with food, especially at high particle densities. This affects respiration and ingestion, eventually resulting in decreased survival rates. Once animals reach the preadult stage, best production yields are obtained by gradually switching from a transparency controlled food distribution (see above) to a feeding scheme of about 10% (dry weight feed to live weight *Artemia*) per day distributed on a semi-continuous basis (every 5 min).[82]

Overfeeding can be prevented by the installation of a simple alarm system which controls

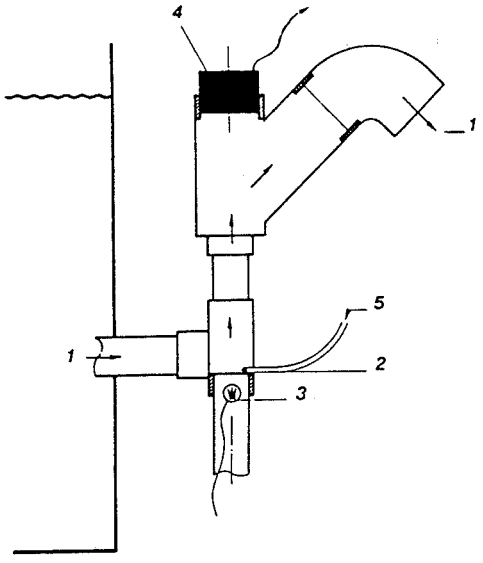

FIGURE 11. Schematic view of electronic transparency meter connected to the outlet of a super-intensive flow-through *Artemia* culture. (1) drain of culture water effluent; (2) transparent PVC-plate; (3) light bulb; (4) light receptor connected to electronic detector; (5) air injection. (From Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E., and Sorgeloos, P., *J. World Aquacult. Soc.*, 16, 498, 1985. With permission.)

the water flow to the culture tanks eventually deactivating the automatic food distribution in case of reduced flow rates.

Recently, a very simple semi-flow-through system has been successfully developed by Bombeo et al.[83] The low flow rates applied (retention times of minimum 12 h) are easily adjusted by means of a siphon with a selected diameter which drains the stock tank by gravity. This latter tank holds culture water and food, and needs to be filled only once a day. This technique involves minimal sophistication and appears to be very predictable in production yields which are in between those obtained in batch and flow-through systems.

B. CLOSED SYSTEMS FOR FLOW-THROUGH CULTURING

When only limited quantities of warm sea water are available, high-density flow-through culturing of *Artemia* can be maintained via a recirculation unit. Although there has been considerable research for developing recirculation systems, the construction of a predictable culture system for marine organisms in closed circuits today is still more an art than a science.[84-85] The following description is an example of an operational recirculating system for *Artemia* culture effluent, as developed at the ARC[75] (Figure 12).

First, the effluent is drained into a small decantation tank (120-1) in which a spiral flow pattern is created by a rolled PVC-plate. After this initial purification step, water flows into a rotating biological contactor (RBC) for biological treatment, i.e., bacterial degradation of soluble organic wastes via oxidative deamination, and nitrification into nitrate. Biodisc purification has been selected for our pilot installation because of its stable operation under conditions of fluctuating hydraulic and organic loadings. It consists of three units of 40 sand-blasted PVC-discs (100 cm in diameter) which rotate at 6 rpm in the interconnected

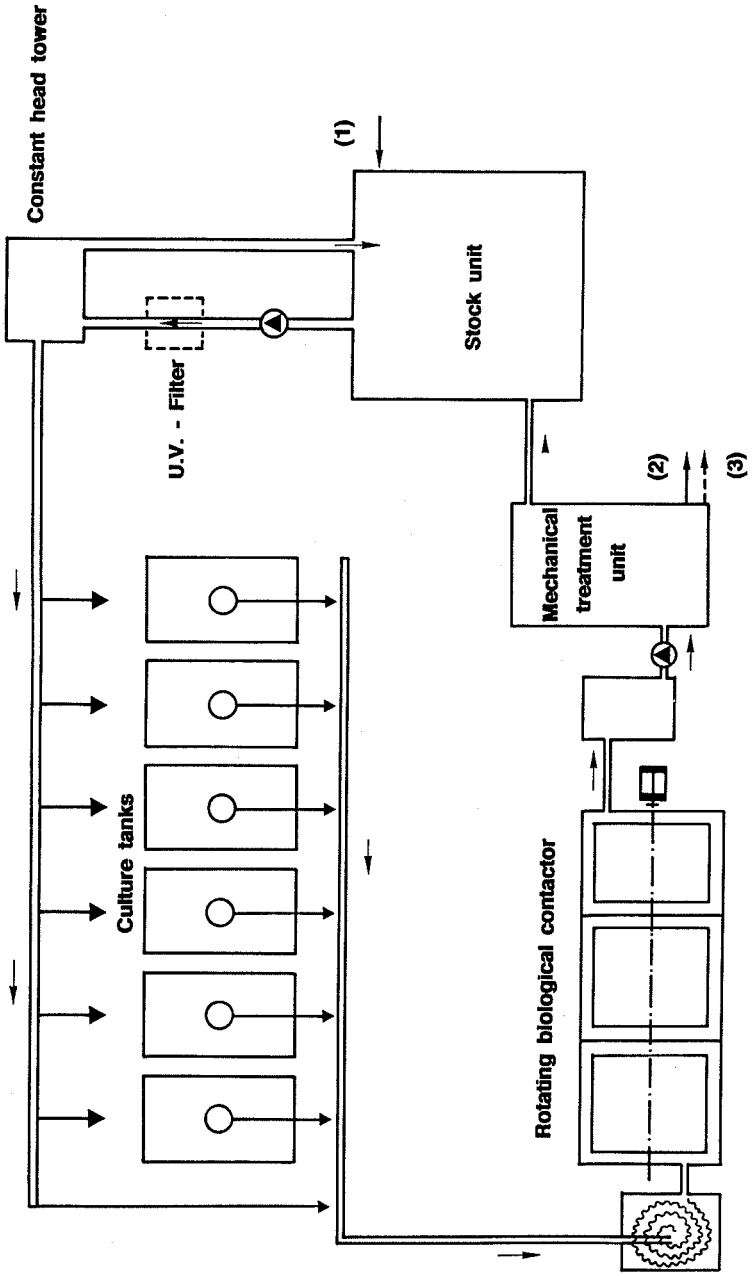

FIGURE 12. Schematic diagram of set-up for closed flow-through culturing of *Artemia*. (1) fresh seawater addition; (2) drain of water; and (3) suspended solids. (From Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E., and Sorgeloos, P., *J. World Aquacult. Soc.*, 16, 498, 1985. With permission.)

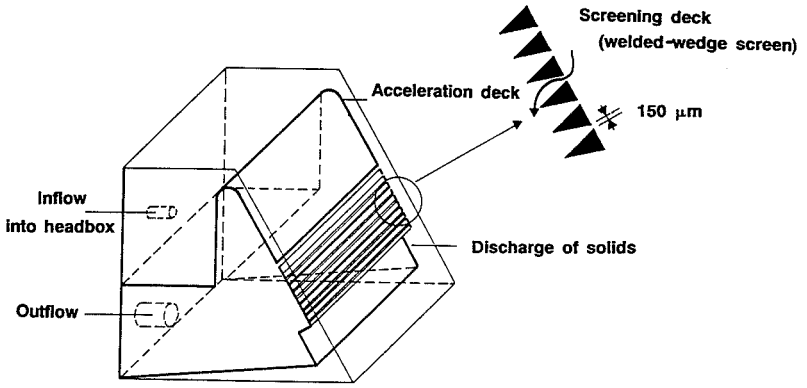

FIGURE 13. Cross-flow sieve system for concentrating suspended particles from *Artemia* culture effluent. (Modified from Bossuyt, E., and Sorgeloos, P., *The Brine Shrimp Artemia*, Vol. 3., Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 133. With permission.)

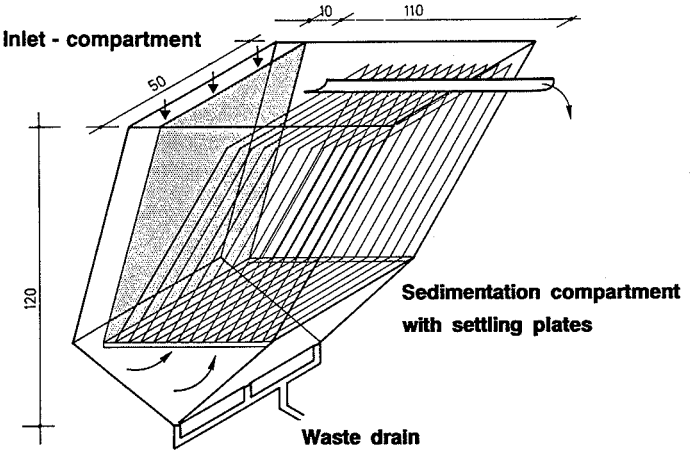

FIGURE 14. Schematic view and dimensions (in cm) of plate separator (650 l) used for primary treatment of 2 m^3 *Artemia* raceway. (Modified from Sorgeloos, P., Lavens, P., Léger, Ph., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, Artemia Reference Center, State University of Ghent, Belgium, 1986. With permission.)

compartments. Total effective surface for bacterial growth is 190 m^2 which is more than sufficient for the purification of 1800-l of culture water with an organic load of about 30 mg/l BOD_5.

The biodisc effluent is pumped over a cross-flow sieve[77] (200 cm^2) with a slit opening of 150 µm (Figure 13). The concentrated sludge is subsequently drained in a plate separator, while the purified water is directly returned to the reservoir. In this way the volume of the plate separator could be reduced 650-1. Its construction is a modification of the plate separator developed by Mock et al.[86] and Bossuyt and Sorgeloos.[77] A schematic drawing and the characteristics of the decantor system are given in Figure 14. It consists of an inclined tank subdivided into a small inflow and a large settling compartment interconnected by an open

space at the conical-shaped bottom part. In the settling compartment 20 sand-blasted PVC-plates with a total surface area of 5.54 m^2 are mounted in an inclined position (60°) oriented in the direction of the water flow through the separator tank. A drain fixed on top of the plates finally evacuates the clean water to the stock tank.

An optimal retention time of ~15 min assures maximal sedimentation of the waste particles. Once every three days the accumulated sludge should be drained by siphoning from the bottom of the decantor.

A last step in the purification process may be disinfection. We installed a UV unit, 4 × 50 W. However, there is no absolute proof of its effectiveness in the culture of *Artemia* (or other organisms), nor does it result in a completely sterile medium.[87] Finally, part of the water should be renewed at regular time intervals. In our system we apply an arbitrarily choosen 25% culture water exchange on a weekly basis.

Using this recirculation system for our *Artemia* culture unit of six 300-l tanks, water quality could be maintained at acceptable levels; i.e., the biological and mechanical treatments result in an effluent containing less than 5 ml/l BOD_5[75] and 22.5 mg/l TOC[3], which accounts for a removal percentage >80%. The levels for NH_4^+-N and NO_2^--N remained <10.9 ppm and 0.6 ppm, respectively, which is far below the tolerance limits for *Artemia*.[3] Levels for suspended solids did not exceed 380 mg/l.

C. STAGNANT CULTURE SYSTEMS

Of the various techniques that have been tested for growing brine shrimp larvae in stagnant culture systems, the air-water-lift operated raceway has proven to be the most suitable.[77] With the exception of the water exchange, the same culture conditions can be maintained as described for flow-through systems. However, automation is more difficult, and monitoring, especially with regard to feeding conditions, more critical.

The first days of culture do not pose any problem and optimal feeding levels can easily be maintained. However, on the fourth day the water quality deteriorates quickly and waste particles — e.g., fecal pellets, food aggregates, and exuviae — physically hamper food uptake by the *Artemia* and interfere with the transparency measurements. The only solution is to install a primary treatment by means of a plate separator (see above) connected to the raceway. This also implies the use of filters to retain the *Artemia* in the culture tanks. Clogging of these filters is less problematic here than in flow-through culturing since no extra water is added and not overflow risks exist. Flow rates and plate separator dimensions are function of the volume of the raceway; optimal particle removal is assured when the culture medium passes over the plate separator at least eight times a day.

Besides the elimination of particulate wastes, it may also be desirable to partially remove the soluble fraction from the culture medium, especially when feeding products that are rich in proteins or which contain a high fraction of soluble material. An easy technique to incorporate in the batch culture design is foam fractioning.[88] A schematic drawing of the foam tower that we used for the final treatment of the effluent of a 2 m^3 raceway is provided in Figure 15.

IV. CONTROL OF INFECTIONS

When experimenting with micronized agricultural by-products as feed for *Artemia* cultures we occasionally found heavy losses of preadults due to infections with filamentous *Leucothrix* bacteria.[3,75] According to Solangi et al.[89] these infections especially occur in nutrient rich media. The *Leucothrix* colonies fix on the exoskeleton, by preference on the thoracopods, and only become visible from the Instar V/VI stage onwards. The brine shrimp suffer physically, as the movements of their thoracopods become affected and filtration rates consequently are reduced. At the upper limit, growth and molting are arrested, overfeeding of the tanks occur, resulting in a collapse of the *Artemia* culture.

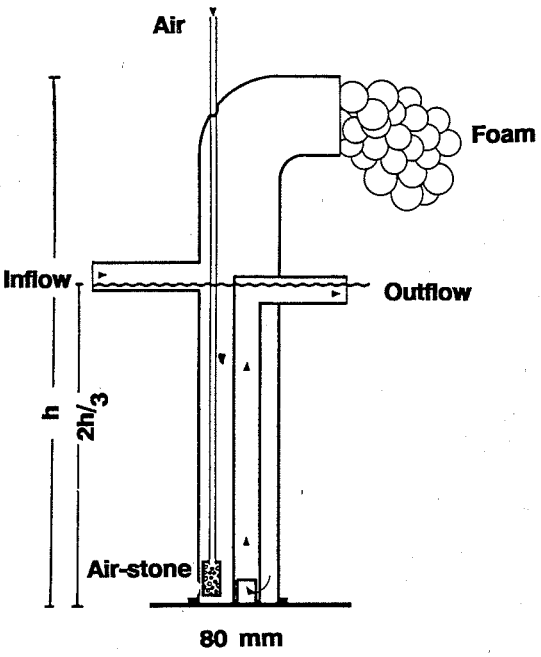

FIGURE 15. Schematic diagram of a foam separator. (From Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, Artemia Reference Center, State University of Ghent, Belgium, 1986. With permission).

Solangi et al. recommend the application of terramycin;[89] however, antibiotics cannot be used in recirculation systems as they will affect the biological treatment unit. We have explored other techniques that are frequently used in urban waste water treatment to eliminate filamentous microorganisms. Frequent additions of low doses of peroxide (up to 50 ppm H_2O_2) proved to be effective in reducing or eliminating the *Leucothrix* populations without affecting the brine shrimp. The most practical solution appeared to be an increase in salinity from 35 to 50—60 g/l together with a higher water renewal rate of 25% instead of 10% on a weekly basis.[3,75]

A second observed disease in *Artemia* cultures is the so-called "black disease"; i.e., part of the animals show black spots, especially at their extremities (thoracopods and antennae). (See Figure 16). According to Hernandorena[90,91] this disease consists of the detachment of the epidermis from the cuticula, and is caused by a dietary deficiency which interferes with the lipid metabolism. In high density culturing of *Artemia* using agricultural by-products as food source, we observed the black disease when water quality deteriorates (probably interfering with the composition of the bacterial population and consequently the diet composition) and/or when feeding rates are not optimal. Improving these conditions doesn't save the affected animals but appears to avoid further losses.

V. PRODUCTION YIELDS

A. HARVESTING AND PROCESSING TECHNIQUES

Harvesting of high-density cultures of *Artemia* can be facilitated by taking advantage of the surface respiration behavior of the animals. When the aeration in the culture tank,

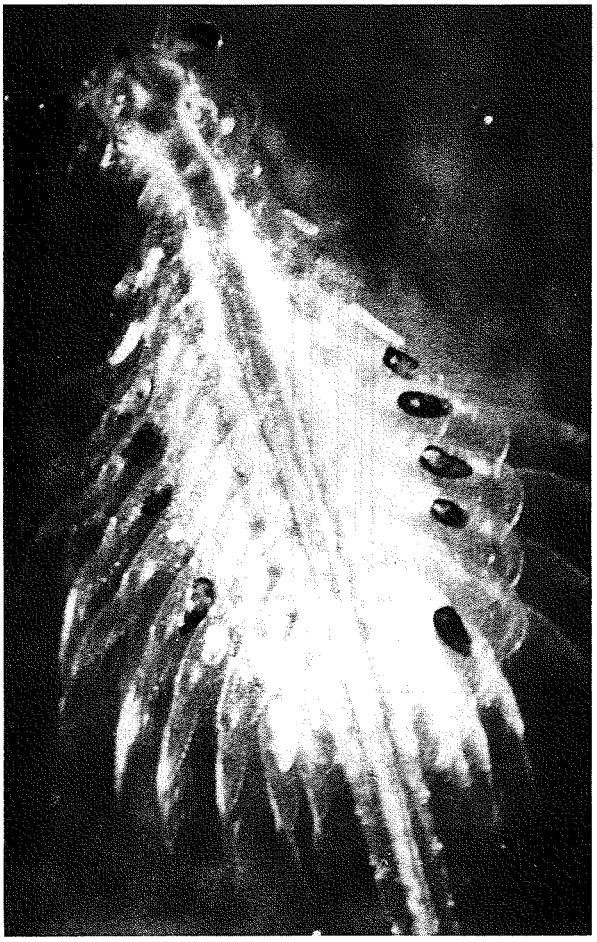

FIGURE 16. Microscopic view of *Artemia* preadult with "black disease" spots on its thoracopods.

together with the flow-through and the automatic feeding are interrupted, oxygen levels in the water drop quickly and all waste particles sink to the bottom; after about 30 min the *Artemia* respond to the oxygen depletion by concentrating at the water surface where they perform surface respiration (i.e., moving the thoracopods at the interface air-water). The concentrated population, free from suspended solids, can easily be scooped with a net of an appropriate mesh size. At low animal densities brine shrimp can be harvested by draining the complete culture over a sieve which should be partially submerged under water.

After a thorough wash in fresh water or sea water, the harvested *Artemia* can be offered as a highly suitable live food for freshwater, as well as marine predators. The salinity of the culture water is of no concern as *Artemia* are hypo-osmoregulator, i.e., their body fluids have a constant and low-salt content of about 9 g/l, as a result of which they are an acceptable food for freshwater animals as well. Furthermore, when transferred into freshwater *Artemia* will continue to swim for another 5 h after which time they eventually die as a result of osmoregulatory stress. In sea water, they remain alive without feeding for several days.

Transportation of live brine shrimp can be done in plastic bags containing cooled sea water and inflated with oxygen. Details about this technique as well as the freezing of *Artemia* biomass are described in Chapter 12 on pond production.

B. PRODUCTION YIELDS

Figure 17 provides a summary of average production data expressed in *Artemia* survival and length, obtained in the different culture systems described in this chapter. After 2 weeks of culturing, preadult or adult *Artemia* with an average length of 5 mm or more can be harvested. Under flow-through culture conditions significant mortality occurs in the first week, probably because the early naupliar stages are more sensitive than the juvenile or preadult stages. This mortality can be reduced by offering a better food, e.g., when feeding live *Chaetoceros* in the St. Croix upwelling system, almost no mortality occurred during the initial culture period.[45,78] No significant differences in survival are noticed between open or closed (with recirculation of the culture medium) flow-through cultures. In stagnant culture installations, on the other hand, survival decreases continuously into the second week which can be explained by the deterioration of the water quality.

Average production yields in terms of ww biomass harvested after 2 weeks culture, amount to 5 kg, 15 kg, and 25 kg *Artemia*/m^3 of tank volume for batch production[73] and flow-through systems using micronized feeds,[74,75] or live algae, respectively.[45] These differences in production figures are primarily the result of differences in maximum stocking density at the start, and final survival at the end of the culture trial.

VI. NUTRITIONAL QUALITY AND USE OF TANK-PRODUCED *ARTEMIA* JUVENILES AND ADULTS IN AQUACULTURE

The opportunities for feeding ongrown *Artemia* to numerous fish and crustacean larval species are well documented in literature.[25] However, until recently, these applications were never utilized at the industrial level because of the limited availability of live or frozen biomass, its high cost, and its variable quality.[2] The possibilities for constructing intensive and super-intensive production facilities for brine shrimp in or nearby the aquaculture farm have resulted during the last decade in increased interests with regard to *Artemia* biomass. Although tank production of *Artemia* is far more expensive than pond production, its advantages for application are manifold:

- Year-round availability of ongrown *Artemia*, independent of climate or season;
- Specific stages (juveniles, preadults, adults) can be harvested with regard to possible size preferences of the predator;
- Quality of the *Artemia* can be better controlled (e.g., nutritionally and free from diseases).

The nutritional quality of *Artemia* juveniles and adults produced in super-intensive systems is analogous to natural product, except for its lipid content. Compared to Instar I nauplii, the protein content of adult *Artemia*, independent of its rearing conditions or food, is appreciably higher, i.e., 60% instead of 47%, and especially richer in essential amino acids.[16,93] (See Tables 7 and 8.) On the other hand, the lipid profile is a reflection of the diet offered to the *Artemia* cultures; i.e., when the animals are cultured on a HUFA-deficient diet (e.g., all agricultural by-products tested so far) their biomass at harvest will contain only small amounts of the fatty acids 20:5(n-3) and 22:6(n-3) (Table 8). However, this does not restrict their application since high levels of essential fatty acids can easily be attained in the *Artemia* biomass by applying the simple bio-encapsulation technique of Léger et al.[97] i.e., in less than 1 h the digestive tract of the brine shrimp can be filled with a HUFA-

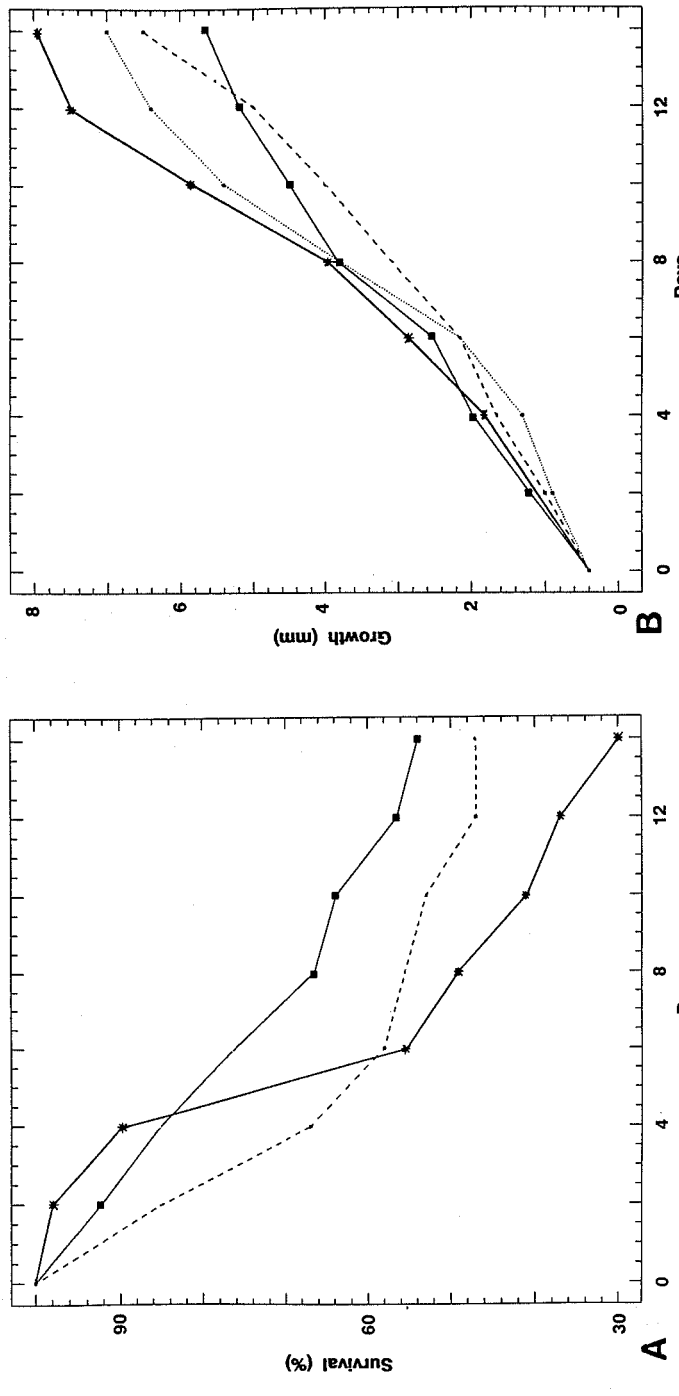

FIGURE 17. Survival (A) and growth (B) of Great Salt Lake *Artemia* cultured in different super-intensive culture systems. ---- 3501 open flow-through culture on rice-bran diet; ..... 2001 open flow-through culture on *Chaetoceras* diet; •——• 3001 closed flow-through culture on mixed diet of soybean waste and corn (maize) bran; *——* 2 m^3 stagnant raceway culture on rice-bran diet. (Modified from Lavens, P., Ph. D. thesis, 1989. With permission.)

TABLE 7
Comparison of the Biochemical Composition of Nauplii and of Preadults Harvested from Super-Intensive Cultures[a]

Artemia characteristics	Proteins	Lipids	N-free extract	Ash	Reference
Instar I - nauplii	41.6 - 47.2	20.8 - 23.1	10.5 - 22.7	9.5	Von Hentig[6], Claus *et al.*[94] Schauer *et al.*[95]
Preadults cultured on corn bran/soybean pellets in a flow-through system	55.2	17.6	10.4	16.8	Abelin[100]
Preadults cultured on rice bran in a stagnant raceway system	56.5	19.5	-	9.0	Dobbeni[96]

[a] All data for the Great Salt Lake strain and expressed in % of dry weight.

enrichment product, e.g. a marine fish-oil emulsion, boosting the HUFA content in the *Artemia* from a low level of 3 mg/g dw up to levels of >16 mg/g. In addition, this enrichment technique offers opportunities to quickly incorporate high amounts of essential nutrients, pigments, or therapeutics in ongrown *Artemia* for specific applications with different predators.

The use of *Artemia* juveniles and adults is not only advantageous from a nutritional point of view but also for energetic reasons; i.e., when feeding on large *Artemia* instead of freshly hatched nauplii, predator larvae need to chase less prey organisms per unit of time to meet their food requirements. This improved energy balance may result in faster growth, a higher developmental rate, and/or an improved physiological condition as has been demonstrated in *Macrobrachium,*[98] *Scophthalmus,*[99] *Sparus,*[100] and *Lates*[101] larviculture. For the latter two species, the introduction of ongrown *Artemia* as a hatchery/nursery food resulted in significant savings of *Artemia* cysts of about 60% and, consequently, a significant reduction in the total larval feed cost.

The aquarium pet shop industry offers expanding marketing opportunities for live *Artemia* biomass produced in regional super-intensive culture systems. Today, over 95% of the more than 3000 metric tons of *Artemia* biomass that are marketed in this sector[2] are sold frozen since they are harvested from a restricted number of natural sources and live transportation to other continents is cost prohibitive. Singapore, for example, already experiences a bottleneck where the local tropical aquarium industry is threatened by a shortage of live foods.[102] In the last decade several super-intensive *Artemia* farms have been established, e.g., in the U.S., France, U.K., and Australia, to supply local demand. Depending on the selected culture technology and implantation facilities, production costs are estimated at 2.5 to 12 US$/kg live weight *Artemia* with wholesale prices varying from $25 to $100.

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TABLE 8
Fatty Acid and Amino Acid Profiles of Great Salt Lake Preadults, Cultured under Flow-Through Conditions on a Mixed Diet of Corn (Maize) Bran and Soybean-Pellets[a]

A. FATTY ACIDS[b]	
saturated	16.20
14:0	0.70
15:0	0.50
16:0	9.10
17:0	0.70
18:0	5.20
19:0	
20:0	
24:0	
unsaturated	46.70
14:1	1.20
14:2	
15:1	0.30
16:1w7 + 16:1w9	4.30
16:2	
16:3	0.30
17:1	
18:1w7 + 18:1w9	18.30
18:2	15.90
18:4	0.10
19:4	
20:1	4.00
21:5	0.30
22:1	2.00
24:1	
unsaturated w3	9.00
18:3	
20:3	0.30
20:4	
20:5	2.80
22:3	0.90
22:4	
22:5	0.40
22:6	4.60
unsaturated w6	0.40
18:3	
20:3	
20:4	
22:4	0.40
22:5	

B. AMINO ACIDS[c]	
essential	26.94
tryptophan	
lysine	4.23
histidine	1.30
arginine	2.69
threonine	2.42
valine	3.20
methionine	0.71
isoleucine	2.96
leucine	4.52
tyrosine	2.16
phenylalaline	2.75
non essential	25.71
asparagine	5.82
serine	2.63
glutamine	7.54
proline	3.29
glycine	2.68
alanine	3.61
cysteine	0.14

[a] Data compiled from Abelin.[100]

[b] Values expressed in mg/g dry matter.

[c] Values expressed in g/100 g dry matter.

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